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A PECULIAR ENTOMOPHTHOROUS FUNGUS

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The writer has for some time been interested in the collection and study of fungi which are found under conditions of a more or less aquatic nature. In this connection a study has been made of the fungi found on fern prothallia grown in water cultures or on moist sphagnum. Both the sphagnum and liquid cultures were carefully sterilized before the sowing of the spores took place.

Among the fungi which from time to time made their appearance in these cultures was one which seemed to all appearances to be a vigorous parasite, and an effort was at once made to isolate it for further study and identification. Thaxter's Potato-hard agar, to which had been added a small quantity of Lofflund's Malt Extract, was poured into sterile Petri dishes and dilution transfers were then made, giving in two days a vigorous growth of the fungus. Pure cultures were easily obtained as it was found that the spores of the fungus were thrown to a considerable distance and by means of the binocular microscope it was possible to pick out individual spores which could then be transferred to test tubes containing media and in a few days a number of pure cultures were available.

The preliminary study seemed to indicate that the fungus was of an Entomophthorous nature and an effort was then made to find the insect upon which it may have been growing. No infections were secured upon any of the insects found in the fern cultures nor upon any of the various insects found in the greenhouse. No successful infections were secured when vigorous fern cultures were inoculated, but it was found that the fungus would grow on dying fern prothallia and upon prothallia which were infected by other fungi. The evidence so far collected seems to indicate that the fungus, altho seem-

ingly very much like Empusa in morphological characteristics, is of a decided saprophytic nature as it has been possible to grow it on many of the common fungal media such as potato-hard agar, oatmeal agar, rice agar, pumpkin agar, but best results have been obtained on agar containing the Malt-soup or a beef extract.

Other investigators have observed a saprophytic condition in members of the Entomophthorales. Mr. Torrey of the Storrs Experiment Station, Storrs, Connecticut, has made a study of such a fungus which he has described in a paper, I believe now in print. Molliard (13) describes *E. henrici* originally obtained as a parasite on *Culex pipiens*, which he has found capable of growing on the sterilized grub of *Euchelia jacobaeae*, on sterilized ox-liver and even on vegetable material such as sterilized carrot. It was found, however, that the growth on the vegetable substratum was not as vigorous as that obtained on animal tissue. These observations indicate that it may well be possible that other members of the group may be able to live at least in part as saprophytes.

The Fungus

The mycelium grows very rapidly and at the end of from 48 to 72 hours forms a thin, compact growth on the surface of the medium. No haustoria or rhizoidal growth has been found and there is very little penetration of the substance upon which the fungus may be growing. The hyphae branch profusely and soon become septate with cells of varying length. The individual cells compare favorably with those described by Thaxter (19) and Olive (16) for Empusa, but contain a greater number of vacuoles and are not as a rule as irregular in shape (Fig. 1). The cytoplasm is of a very heavy granular nature and does not as a rule contain the conspicuous fat bodies described by these authors. This of course may in part be due to the fact that the media used differ greatly in food content from the bodies of insects upon which the Empusas grow. Fat or oil bodies are more often found in the older hyphal cells and in the mature conidia.

The shape and size of individual cells vary greatly, dependent upon the nature of the medium upon which the fungus is growing. On a medium poor in food material the cells are more elongate and

narrow in diameter, while on a rich substratum they are thicker, shorter, and less vacuolate.

Reproduction

At the end of from 36 to 48 hours the conidiophores begin to make their appearance in considerable numbers. These may arise from any portion of the hyphae, but usually arise from the terminal cells. Search has been made for the hyphal bodies described by Thaxter (19) and Olive (16) but no cells quite comparable to these have been found, although it is evident at times that the cells which give rise to the conidiophores are filled with a denser cytoplasm and are often more irregular in shape than neighboring cells. Nothing comparable to the sclerotia described by Sorokin (17, 18) have been found in any of these cultures.

The conidiophores arise as branches from the cell and are usually simple, each producing a single conidium (Figs. 2, 3), but many cases of compound conidiophores are found, in some instances quite comparable to those described by Thaxter for *Empusa occidentalis* and *Empusa Aphidis* (Figs. 6-9).

The conidiophore becomes club-shaped and filled with a very heavy granular cytoplasm which at once distinguishes it from any of the cells of the vegetative hyphae. The apical portion soon expands into a "basidium" which finally reaches about the diameter of the mature conidium. By this time the basal portion of the conidiophore has become greatly vacuolated and there seems to be a decided movement of the cytoplasm into the rounded upper end. It has not been possible to definitely decide just what takes place during the next stage but a columella like structure soon makes its appearance, cutting off the enlarged portion of the basidium, and within this cell a membrane is laid down to form the wall of the single spore. Figs. 5, 10, 11.

A portion of the content of the basidium continues to pass into the maturing conidium which finally becomes filled with a very dense granular cytoplasm, in which appear at times a few fat bodies. During this later stage the portion of the conidium which is in contact with the "columella" becomes decidedly conical with the apex of the cone usually pressed against the columella. The cytoplasm within the basidium at this stage is decidedly hyaline and at times appears to contain little or no cytoplasm.

The process by which the basidium ruptures and projects the conidium is not fully understood. Many instances have been found where it appears as if the pointed portion of the conidium tends to weaken or pierce the columella of the basidium which is continually becoming more turgid, due to the intake of water, and finally the pressure becomes so great that the entire end of the basidium is torn asunder, thus discharging the conidium with such force that it is often thrown to a distance of 65 mm.

A portion of the content of the basidium is probably thrown with the conidium and it seemingly is due to this material that the conidium will readily adhere to any substance with which it may come in contact. Although this seems to be the usual procedure, other cases have been observed where it appears as if the basidial wall ruptures without any damage to the columella.

The Conidia

The mature conidium is perfectly spherical except for a conspicuous "appiculus" found at the point of contact with the columella. The cytoplasm is usually quite dense, finely granular, with an occasional fat body. The primary conidia have a diameter varying from 48μ–60μ, the secondary conidia average 35μ–40μ, while the tertiary conidia at times have a diameter of not more than 20μ. The secondary and tertiary conidia are often characterized by the presence of a very large vacuole and conspicuous fat bodies are at times also apparent.

Germination of the Conidia

When a conidium falls upon a substratum containing some moisture, it germinates in from 6–12 hours, putting out from one to four germ tubes which in a very short time develop into the typical septate mycelium previously described. Fig. 16. If, however, a conidium falls upon a surface free from moisture, it at once develops a very short tube, often less than one-tenth as great as the diameter of the spore, at the end of which is produced a secondary conidium averaging about two-thirds the diameter of the primary spore. Figs. 18, 22. This secondary conidium is formed like the primary spore and is discharged in the same manner, and may now produce either a mycelium or a "tertiary" spore, dependent upon the nature of the substratum.

In some cultures it has been found that the primary conidia do not germinate if discharged upon an unfavorable substance, but instead there appears a slight thickening of the spore wall, while at the same time the contents become decidedly yellowish in color. These were at first thought to be resting spores but it has so far been impossible to obtain any evidence of germination.

In a few rare cases the germ tubes continue to elongate for a time and then produce a basidium and a secondary conidium. In such cases the secondary conidium is of a smaller size than the normal secondary spore, probably due to the fact that much of the content of the primary spore was used in formation of the hyphae. Figs. 19, 20.

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EXPLANATION OF PLATES (XXVII—XXVIII)

All figures were drawn with the aid of the camera lucida, with a Leitz No. 3 ocular and Leitz No. 6 objective.

Plate XXVII

Fig. 1. Typical cells from mycelium grown on agar containing beef extract.
Fig. 2. Early stage in formation of conidiophore.
Fig. 3. Conidiophore separated from balance of mycelium by cell wall.
Fig. 4. Coniodiophore with dense cytoplasm gathered at apex which is beginning to increase in size.
Fig. 5. Terminal portion of conidiophore fully enlarged and "columella" partly completed.
Figs. 6, 7, 8, and 9. Abnormal types of conidiophores which however produce typical conidia.

PLATE XXVIII

Fig. 10. Upper portion of conidiophore, showing "columella" and also indications of spore wall.
Fig. 11. Conidial wall completely laid down. At this stage the columella usually forms an indentation in the maturing spore.
Fig. 12. Slightly later stage. The "appiculus" is in process of development and has forced the columella backward into the basidium.
Figs. 13 and 14. Fully matured conidia still attached to basidium which at this stage contains a very small amount of cytoplasm.
Fig. 15. Primary conidium showing heavy granular appearance.
Fig. 16. Germination of primary spore sown on agar containing Malt-soup extract.
Fig. 17. Germination of primary spore sown on potato-hard agar (sown at same time as spore in Fig. 16.)
Fig. 18. Early stage in formation of secondary spore.
Figs. 19 and 20. Abnormal formation of secondary spore. In each case a conidiophore has been formed.
Fig. 21. Germination of secondary spore.
Fig. 22. Formation of tertiary spore by secondary spore.
Fig. 23. Germination of tertiary spore.